Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	3	("5324637" or "6168931" or "5665563").pn.	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 14:36
S2	201768	transcription or translation or protein synthesis	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 14:37
S3	9322	S2 near5 (in vitro or cell free)	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 14:38
S4	1418	nuclease near3 inhibit$	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 14:39
S5	1604	ribonuclease$ near3 inhibit$	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 14:40
S6	47	S3 same (S4 or S5)	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 16:22
S7	292	recBC$ or exov or exo adj v	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 16:24
S8	11	S3 and S7	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 16:24

priority to 3/8/01

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	3	("5324637" or "6168931" or "5665563").pn.	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 14:36
S2	201768	transcription or translation or protein synthesis	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 14:37
S3	9322	S2 near5 (in vitro or cell free)	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 14:38
S4	1418	nuclease near3 inhibit$	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 14:39
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S6	47	S3 same (S4 or S5)	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 16:22
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S8	11	S3 and S7	US-PGPUB; USPAT	ADJ	OFF	2004/12/02 16:24

PGPUB-DOCUMENT-NUMBER: 20040235029

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040235029 A1

TITLE: In vitro translation system

PUBLICATION-DATE: November 25, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 832820

DATE FILED: April 27, 2004

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60465963 20030428 US

US-CL-CURRENT: 435/6, 435/7.1

ABSTRACT:

In vitro translation (IVT) systems and methods for increased expression of proteins from linear templates, using GamS, are provided. The proteins may be full length or protein fragments. The IVT system may be used in batch or continuous mode. The GamS may be used as GamS nucleic acid template, crude protein fraction, or purified protein product. The IVT system using GamS component may be employed in a high-throughput mode. The ability to predict expressible protein or fragments, and activity and solubility of a large-scale protein expression product based on the results obtained from high-throughput, small-scale IVT expression product is also provided.

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional patent application 60/465,963 filed Apr. 28, 2003. The contents of the prior application are hereby incorporated in their entirety.

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Abstract Paragraph - ABTX (1):

In vitro translation (IVT) systems and methods for increased expression of proteins from linear templates, using GamS, are provided. The proteins may be full length or protein fragments. The IVT system may be used in batch or continuous mode. The GamS may be used as GamS nucleic acid template, crude protein fraction, or purified protein product. The IVT system using GamS component may be employed in a high-throughput mode. The ability to predict

expressible protein or fragments, and activity and solubility of a large-scale protein expression product based on the results obtained from high-throughput, small-scale IVT expression product is also provided.

Pre-Grant Publication Document Identifier - DID (1):
US 20040235029 A1

Title - TTL (1):
In vitro translation system

Summary of Invention Paragraph - BSTX (2):
[0002] In vitro translation (IVT), a cell-free method of protein expression, is an attractive alternative to the conventional in-vivo technologies for protein production such as bacterial fermentation and cell culture. Some advantages IVT has over cell-based systems are: 1) it allows direct access to reaction conditions; 2) it is free of all cell functions except protein production; 3) the products of the synthesis do not affect continued productivity; and 4) it is simpler, faster, and suitable for high-throughput expression systems. The nucleic acid that encodes the protein to be expressed is referred to as a "template". Templates for IVT may be circular (inside plasmids, for example) or linear. Use of linear templates for IVT is advantageous over the use of circular templates, since linear templates can be made directly by PCR, thus avoiding many laborious steps such as subcloning, transformation, plasmid isolation, and sequencing. Therefore, IVT using linear templates is ideal for making a large number of different proteins in high-throughput mode as well as screening many different constructs or mutants of given genes. However, one drawback of IVT using linear templates is low protein yield when used in conjunction with E. coli extracts, mainly due to the degradation of linear DNA by exonuclease V, or ExoV of E. coli (see Pratt J M (1984) and references therein). ExoV, a component of RecBCD holoenzyme, harbors both ATP-dependent 3'- and 5'-exonuclease activities, and digests both single- and double-strand DNA. Several attempts have been made to improve the protein yield from linear templates by avoiding the ExoV activity. For example, ExoV mutant strains have been used to make extracts, however, those mutants grow poorly and extracts are contaminated with large amounts of host chromosomal DNA (Gold and Schweiger (1972); Jackson et al (1983); Yang et al (1980); Yu et al (2000)). As another example, temperature sensitive ExoV mutants have also been used such that extract is prepared at a temperature in which ExoV is active, and IVT reaction is done at a high temperature in which ExoV is inactive. Still, the limitation of the IVT reaction only at high temperature is a problem (Jackson et al (1983)). As yet another example, cell extracts have been fractionated to remove the exonuclease, however, the reproducibility and efficiency of quality of extract are problematic.
Therefore, an improved IVT system with enhanced capability of producing protein from linear templates would be desirable for providing increased protein yield for research and drug discovery.

Summary of Invention Paragraph - BSTX (3):
[0003] Bacteriophage lambda is known to carry a gene that inhibits the ExoV activity of a host cell. The gene, called "Gam" for gamma, is expressed at the late stage of the phage cycle and prevents its genomic linear DNA from degradation by ExoV before packaging into the phage particles (Karu et al (1975)). The Gam gene encodes a protein, referred to as "GamL", which is 138 amino acids long and has a predicted molecular weight of 16,349 daltons. It has been purified from E. coli, and been shown to inhibit ExoV activity by binding directly to the enzyme, not DNA (Karu et al , supra). A shorter form of the Gam protein, referred to as "GamS" having the gam activity by genetic means has also been reported (Friedman and Hays (1986)). GamS lacks the

N-terminal 40 amino acids due to translation initiation at an internal, in frame, ATG of the Gam gene. This results in the smaller GamS of 98 amino acids, and 11646 daltons. GamS exhibits all activities associated with a GamL protein in cells. However, to date, due to lack of purified GamS, it has not been determined which Gam protein (GamL, GamS, or both) is the functional protein having ExoV inhibition activity.

Detail Description Paragraph - DETX (2):

[0007] The invention provides an in vitro transcription/translation (IVT) system and method for linear templates comprising a GamS component. The GamS component may be in the form of a GamS-encoding nucleic acid or protein. The IVT system may operate in batch or continuous mode. The IVT system may be employed in a high-throughput manner to provide simultaneous protein expression from an array of linear templates. In various alternative embodiments, the expressed protein is a full-length protein, or a protein fragment, such as a protein domain or subdomain, or a fusion or chimeric protein, among others. GamS inhibits the ExoV activity of E. coli, thus dramatically increasing the yield of the expressed protein as compared with an IVT method or system that does not employ GamS. The utility of the invention is the increased yield of the expressed protein, which, in turn, is useful in protein research and drug discovery applications, such as parallel protein synthesis, optimization of expression constructs, functional testing of PCR generated mutations, expression of truncated proteins or protein fragments for epitope or functional domain mapping, full length protein and protein domain crystallization for structural biology applications, and expression of toxic gene products, among others. An unexpected additional utility of the invention is that results of protein expression in small quantities using GamS allow prediction of protein solubility and activity for large-scale expression of the same protein. Various alternative large-scale expression systems such as baculovirus, E. coli, IVT, and mammalian systems, among others, may be employed for large-scale protein productions. Thus, the invention additionally provides methods for alternating between various protein production methods when switching between a small-scale and a large-scale expression system.

Detail Description Paragraph - DETX (5):

[0010] The linear template may be transcribed as part of the IVT system, or prepared prior to additon to the IVT system. Transcription of DNA can occur in vivo or in vitro, from prokaryotic or eukaryotic cells or cell extracts, prior to in vitro translation. In vivo transcription systems are difficult to work with, since intact cells are used. In vitro transcription systems for both prokaryotic and eukaryotic systems are commercially available, and well known in the art. In vitro translation systems that are made from prokaryotic cells such as E. coli, or from eukaryotic cells such as rabbit reticulocyte and wheat germ, or from DNA sequences cloned into a vector containing an RNA polymerase promoter are also well known in the art (Zubay (1973); Pelham (1976); Roberts (1973); Krieg P (1984)).

Detail Description Paragraph - DETX (37):

[0041] The purified GamS protein of Example II was added to the RTS.TM. 100HY reaction mixture (batch mode) containing the linear PCR template of the GFP to test the stimulatory activity of GamS protein. A linear template was made for the green fluorescent protein (GFP) with a C-terminal His tag and used as an example. The typical concentration of the GFP linear template was 2 to 5 .mu.g/ml in the final reaction. Typically, the GamS protein was added to the reaction mixture and incubated for 20 minutes on ice before adding the GFP linear template. Since GamS binds and blocks ExoV, it was added into the reaction prior to addition of the DNA template. GamS might be added along with or even after addition of nucleic acid template, but in these cases some nucleic acid might be digested before ExoV inhibition activity of GamS, thus

resulting in reduced yield of the resulting protein product. The following GamS concentrations were used in the experiments: 0.5, 1, 2, 5, and 10 .mu.g/ml. Coomassie staining of the gel for reaction products indicated that GFP protein synthesis was increased notably for each GamS concentration as compared with control reactions lacking GamS, and was approximately three fold at 2 .mu.g/ml of GamS. Concentrations larger than 2 .mu.g/ml of GamS resulted in slight further increase in GFP protein synthesis. To confirm that GamS can increase the expression of proteins other than GFP, the GamS protein was tested on expression of three other proteins (protein kinases) from linear templates. The GamS was added at 2 .mu.g/ml in these experiments, and increased protein expression for all of the proteins. These data clearly demonstrate that the GamS protein enhances protein yield in linear-template-based IVT for various proteins.

Detail Description Paragraph - DETX (44):

[0048] Taken together, these experiments indicate that GamS, via inhibition of the ExoV activity of E. coli, dramatically improves protein yields in IVT systems using linear templates as compared with systems lacking GamS. The IVT systems may be in batch or continuous mode. To enhance protein expression, GamS can be used as a co-expressed template, as a crude fraction, or as purified protein. Further, GamS may be used in any other systems that require protection from prokaryotic exonuclease activity.

Detail Description Paragraph - DETX (264):

[0268] Friedman, S A. and Hays, J. B. Selective Inhibition of Escherichia coli recBC activities by plasmid-encoded GamS function of phage lambda. Gene 43, 255-263, 1986.

Detail Description Paragraph - DETX (270):

[0274] Kim, D. M. and Swartz, J. R. Regeneration of adenosine triphosphate from glycolytic intermediates for cell-free protein synthesis. Biotechnology and Bioengineering 74, 309-316, 2001.

Detail Description Paragraph - DETX (273):

[0277] Pratt, J. M. Coupled Transcription-Translation in Prokaryotic Cell-Free Systems. p179-209. In "Transcription and Translation: Practical approach" Edited by Hanes B. D. and Higgins, H. J. 1984.

Detail Description Paragraph - DETX (275):

[0279] Spirin, A. S., Baranov V. I., Ryabova L. A., Ovodov S. Y., and Alakhov Y. B. A continuous cell-free translation system capable of producing polypeptides in high yield. Science 242, 1162-1164, 1988.

Detail Description Paragraph - DETX (277):

[0281] Yang H L, Ivashkiv L, Chen H Z, Zubay G, Cashel M. Cell-free coupled transcription-translation system for investigation of linear DNA segments. Proc Natl Acad Sci USA. 1980 December;77(12):7029-33.

PGPUB-DOCUMENT-NUMBER: 20040077090

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040077090 A1

TITLE: Whole cell engineering by mutagenizing a substantial portion of a starting genome, combining mutations, and optionally repeating

PUBLICATION-DATE: April 22, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 383798

DATE FILED: March 6, 2003

RELATED-US-APPL-DATA:

child 10383798 A1 20030306

parent continuation-of 09677584 20000930 US ABANDONED

child 09677584 20000930 US

parent continuation-in-part-of 09594459 20000614 US GRANTED

parent-patent 6605449 US

child 09594459 20000614 US

parent continuation-in-part-of 09522289 20000309 US GRANTED

parent-patent 6358709 US

child 09522289 20000309 US

parent continuation-in-part-of 09498557 20000204 US PENDING

child 09498557 20000204 US

parent continuation-in-part-of 09495052 20000131 US GRANTED

parent-patent 6479258 US

non-provisional-of-provisional 60156815 19990929 US

US-CL-CURRENT: 435/471, 435/252.3 , 435/254.2

ABSTRACT:

An invention comprising cellular transformation, directed evolution, and screening methods for creating novel transgenic organisms having desirable properties. Thus in one aspect, this invention relates to a method of generating a transgenic organism, such as a microbe or a plant, having a

plurality of traits that are differentially activatable. Also, a method of retooling genes and gene pathways by the introduction of regulatory sequences, such as promoters, that are operable in an intended host, thus conferring operability to a novel gene pathway when it is introduced into an intended host. For example a novel man-made gene pathway, generated based on microbially-derived progenitor templates, that is operable in a plant cell. Furthermore, a method of generating novel host organisms having increased expression of desirable traits, recombinant genes, and gene products.

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Pre-Grant Publication Document Identifier - DID (1):
US 20040077090 A1

Detail Description Paragraph - DETX (2885):
[3049] Conjugative transfer is effected by an origin of transfer (oriT) and flanking genes (MOB A, B and C, and 15-25 genes, termed tra, encoding the structures and enzymes necessary for conjugation to occur. The transfer origin is defined as the site required in cis for DNA transfer. Tra genes include tra A, B, C, D, E, F, G, H, I, J, K, L, M, N, P, Q, R, S, T, U, V, W, X, Y, Z, virAB(allelesl-11), C, D, E, G, HF, and FinOP. Tra genes can be expressed in cis or trans to oriT. Other cellular enzymes, including those of the RecBCD pathway, RecA, SSB protein, DNA gyrase, DNA poll, and DNA ligase, are also involved in conjugative transfer. RecE or recF pathways can substitute for RecBCD.

Detail Description Paragraph - DETX (2940):
[3104] This is due, at least in part, to the Exonuclease V (Exo V) activity of the RecBCD holoenzyme which rapidly degrades linear DNA molecules following transformation. Production of ExoV has been traced to the recD gene, which encodes the D subunit of the holoenzyme. As demonstrated by Russel et al. (1989) Journal of Bacteriology 2609-2613, homologous recombination between a transformed linear donor DNA molecule and the chromosome of recipient is readily detected in a strains bearing a loss of function mutation in a recD mutant.

Detail Description Paragraph - DETX (2942):
[3106] The use of RecE/recT as described supra, can improve homologous recombination of linear DNA fragments. The RecBCD holoezyme plays an important role in initiation of RecA-dependent homologous recombination. Upon recognizing a dsDNA end, the RecBCD enzyme unwinds and degrades the DNA asymmetrically in a 5' to 3' direction until it encounters a chi (or `X`)-site (consensus 5'-GCTGGTGG-3) which attenuates the nuclease activity. This results in the generation of a ssDNA terminating near the c site with a 3'-ssDNA tail that is preferred for RecA loading and subsequent invasion of dsDNA for homologous recombination. Accordingly, preprocessing of transforming fragments with a 5' to 3' specific ssDNA Exonuclease, such as Lamda ( ) exonuclease (available, e.g., from Boeringer Mannheim) prior to transformation may serve to stimulate homologous recombination in recD-strain by providing ssDNA invasive end for RecA loading and subsequent strand invasion.

Detail Description Paragraph - DETX (2943):
[3107] The addition of DNA sequence encoding chi-sites (consensus 5'-GCTGGTGG-3') to DNA fragments can serve to both attenuate Exonuclease V activity and stimulate homologous recombination, thereby obviating the need for a recD mutation (see also, Kowalczykowski, et al. (1994) "Biochemistry of homologous recombination in Escherichia coli," Microbiol. Rev. 58:401-465 and

Jessen, et al. (1998) "Modification of bacterial artificial chromosomes through Chi-stimulated homologous recombination and its application in zebrafish transgenesis." Proc. Natl. Acad. Sci. 95:5121-5126). Chi sites are optionally included in linkers ligated to the ends of ttansformiing fragments or incorporated into the external primers used to generate DNA fragments to be transformed. The use of recombination-stimulatory sequences such as chi is a generally useful approach for evolution of a broad range of cell types by fragment transformation. Methods to inhibit or mutate analogs of Exo V or other nucleases (such as, Exonucleases I (endA 1), 111 (nth), IV (nfo), VII, and VIII of E. coli) is similarly useful.

Detail Description Table CWU - DETL (13):

search for similarities in the amino acid sequence of two proteins. J Mol Biol 48(3):443-453, 1970. Nelson M, Christ C, Schildkraut I: Alteration of apparent restriction endonuclease recognition specificities by DNA methylases. Nucleic Acids Res 12(13):5165-73, 1984 (Jul. 11). Nicholls P J, Johnson V G, Andrew S M, Hoogenboom H R, Raus J C, Youle R J: Characterization of single- chain antibody (sFv)-toxin fusion proteins produced in vitro in rabbit reticulocyte lysate. J Biol Chem 268(7):5302-5308, 1993. Oller A R, Vanden Broek W, Conrad M, Topal M D: Ability of DNA and spermidine to affect the activity of restriction endonucleases from several bacterial species. Biochemistry 30(9):2543-9, (Mar. 5) 1991. Owen M R L, Pen J: Transgenic Plants: A Production System for Industrial and Pharmaceutical Proteins. Chichester: John Wiley & Sons, 1996. Owens R J and Young R J: The genetic engineering of monoclonal antibodies. J Immunol Methods 168(2):149-165, 1994. Pearson W R and Lipman D I: Improved tools for biological sequence comparison. Proc Natl Acad Sci USA 85(8):2444-2448, 1988. Pein C D, Reuter M, Meisel A, Cech D, Kruger D H: Activation of restriction endonuclease EcoRII does not depend on the cleavage of stimulator DNA. Nucleic Acids Res 19(19):5139-42, (Oct. 11) 1991. Persson M A, Caothien R H, Burton D R: Generation of diverse high-affinity human monoclonal antibodies by repertoire cloning. Proc Natl Acad Sci USA 88(6):2432-2436, 1991. Perun T J, Propst C L, eds.: Computer-Aided Drug Design: Methods and Applications. New York: Marcel Dekker, Inc., 1989. Qiang B Q, McClelland M, Poddar S, Spokauskas A, Nelson M: The apparent specificity of NotI (5'- GCGGCCGC-3') is enhanced by M.FnuDII or M.BepI methyltransferases (5'-mCGCG-3): cutting bacterial chromosomes into a few large pieces. Gene 88(1):101-5, (Mar. 30) 1990. Queen C, Foster J, Stanber C, Stafford J: Cell-type specific regulation of a kappa immunoglobulin gene by promoter and enhance elements. Immunol Rev 89:49-68, 1986. Raleigh E A, Wilson G: Escherichia coli K-12 restricts DNA containing 5-methylcytosine. Proc Natl Acad Sci USA 83(23):9070-4, (December) 1986. Reidhaar-Olson J F and Saner R T: Combinatorial cassette mutagenesis as a probe of the informational content of protein sequences. Science 241(4861):53-57, 1988. Riechmann L and Weill M: Phage display and selection of a site-directed randomized single-chain antibody Fv fragment for its affinity improvement. Biochemistry 32(34):8848-8855, 1993. Roberts R J, Macelis D: REBASE--restriction enzymes and methylases. Nucleic Acids Res 24(1):223-35, (Jan. 1) 1996. Ryan A J, Royal C L, Hutchinson J, Shaw C H: Genomic sequence of a 12S seed storage protein from oilseed rape (Brassica napus c.v. jet neuf) Nucl Acids Res 17(9):3584, 1989. Sambrook J, Fritsch E F, Maniatis T. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, .COPYRGT. 1982. Sambrook J, Fritsch E F, Maniatis T. Molecular Cloning: A Laboratory Manual. Second Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, .COPYRGT. 1989. Scopes R K. Protein Purification: Principles and Practice. Springer-Verlag, New York, NY, .COPYRGT. 1982. Segel I H: Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems. New York: John Wiley & Sons, Inc., 1993. Silver S C and Hunt S W 3d: Techniques for cloning cDNAs encoding interactive transcriptional regulatory proteins. Mol Biol Rep 17(3): 155-165,

1993. Smith T F, Waterman M S, Fitch W M: Comparative biosequence metrics. J Mol Evol S18(1):38-46, 1981. Smith T F, Waterman M S. Adv Appl Math 2:482-end of article, 1981; Smith T F, Waterman M S: Identification of common molecular subsequences. J Mol Biol 147(1):195-7, (Mar. 25) 1981. Smith T F, Waterman M S: Overlapping genes and information theory. J Theor Biol 91(2):379-80, (Jul. 21) 1981. Standinger J, Perry M, Elledge S J, Olson E N: Interactions among vertebrate helix-loop-helix proteins in yeast using the two-hybrid system. J Biol Chem 268(7):4608-4611, 1993. Stemmer W P, Morris S K, Wilson B S: Selection of an active single chain Fv antibody from a protein linker library prepared by enzymatic inverse PCR. Biotechniques 14(2):256-265, 1993. Stemmer W P: DNA shuffling by random fragmentation and reassembly: in vitro recombination for molecular evolution. Proc Natl Acad Sci USA 91(22):10747-10751, 1994. Sun D, Hurley L H: Effect of the (+)-CC-1065-(N3-adenine)DNA adduct on in vitro DNA synthesis mediated by Escherichia coli DNA polymerase. Biochemistry 31:10, 2822-9, (Mar. 17) 1992, Tague B W, Dickinson C D, Chrispeels M I: A short domain of the plant vacuolar protein phytohemagglutinin targets invertase to the yeast vacuole. Plant Cell 2(6):533-46, (June) 1990. Takahashi N, Kobayashi I: Evidence for the double-strand break repair model of bacteriophage lambda recombination. Proc Natl Acad Sci USA 87(7):2790-4, (April) 1990. Thiesen H J and Bach C: Target Detection Assay (TDA): a versatile procedure to determine DNA binding sites as demonstrated on SP1 protein. Nucleic Acids Res 18(11):3203-3209, 1990. Thomas M, Davis R W: Studies on the cleavage of bacteriophage lambda DNA with EcoRI Restriction endonuclease. J Mol Biol 91(3):315-28, (Jan. 25) 1975. Tingey S V, Walker E L, Corruzzi G M: Glutanine synthetase genes of pea encode distinct polypeptides which are differentially expressed in leaves, roots and nodules. EMBO J 6(1):1-9, 1987. Topal M D, Thresher R J, Conrad M, Griffith J: Nael endonuclease binding to pBR322 DNA induces lopping. Biochemistry 30(7):2006-10, (Feb. 19) 1991. Tramontano A, Chothia C, Lesk A M: Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins. J Mol Biol 215(1):175-182, 1990. Tuerk C and Gold L: Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249(4968):505-510, 1990. U.S. Pat. No. 4,683,195; Filed Feb. 7, 1986, Issued Jul. 28. 1987. Mullis K B, Erlich H A, Arnheim N, Horn G T, Saiki R K, Scharf S J: Process for Amplifying, Detecting, and/or Cloning Nucleic Acid Sequences. U.S. Pat. No. 4,683,202; Filed Oct. 25, 1985, Issued Jul. 28, 1987. Mullis K B: Process for Amplifying Nucleic Acid Sequences. U.S. Pat. No. 4,704,362; Filed Nov. 5, 1979, Issued Nov. 3, 1987. Itakura K, Riggs A D: Recombinant Cloning Vehicle Microbial Polypeptide Expression. U.S. Pat. No. 4,713,337; Filed Jan. 3, 1985, Issued Dec. 15, 1987. Jasin M, Schimmel P R: Method for deletion of a gene from a bacteria. U.S. Pat. No. 4,732,856; Filed Apr. 3, 1984, Issued Mar. 22, 1988. Federoff N V: Transposable elements and process for using same. U.S. Pat. No. 4,963,487; Filed Sep. 14, 1987, Issued Jan. 16, 1990. Schimmel P R: Method for deletion of a gene from a bacteria. U.S. Pat. No. 5,354,656; Filed Oct. 2, 1989, Issued Oct. 11, 1994. Sorge, Joseph A.; Huse, William D.: U.S. Pat. No. 5,385,835; Filed May 19, 1994, Issued Jan. 31, 1995. Helentjaris, Timothy; Nienhuis, James: Identification and localization and introgression into plants of desired multigenic traits. U.S. Pat. No. 5,453,247; Filed Nov. 23, 1993, Issued Sep. 26, 1995. Beavis, Ronald C.; Chait, Brian T.: Instrument and method for the sequencing of genome. U.S. Pat. No. 5,604,100; Filed Jul. 19, 1995, Issued Feb. 18, 1997. Perlin, Mark W.: Method and system for sequencing genomes. U.S. Pat. No. 5,670,321; Filed May 10, 1995, Issued Sep. 23, 1997. Kimmel, Bruce E.; Ellis, Michael; Ruddy, David: Efficient method to conduct large-scale genome sequencing. U.S. Pat. No. 5,925,808; Filed Dec. 19, 1997, Issued Jul. 20, 1999. Oliver, Melvin John; Quisenberry, Jerry Edwin; Trolinder, Norma Lee Glover; Keim, Don Lee: Control Of Plant Gene Expression. U.S. Pat. No. 5,953,727; Filed Mar. 6, 1997, Issued Sept. 14, 1999. Maslyn, Timothy J.;

Au-Young, Janice; Hillman, Jennifer L.; Hibbert, Harold; Akerblom, Ingrid E.; Cheng, Rachel J.; Tang, Yuanhua T.:Project- based full-length biomolecular sequence database. U.S. Pat. No. 5,965,443; Filed Sep. 9, 1996, Issued Oct. 12, 1999. Reznikoff W S, Goryshin I Y: System for in vitro transposition. U.S. Pat. No. 5,981,177; Filed Jan. 25, 1995, Issued Nov. 9, 1999. Demirjian D C, Casadaban M I, Weber M, Gaines G L: Protein fusion method and constructs. U.S. Pat. No. 5,994,058; Filed Mar. 20, 1995, Issued Nov. 30, 1999. Senapathy, Periannan:Method For Contiguous Genome Sequencing. U.S. Pat. No. 6,023,659; Filed Mar. 6, 1997, Issued Feb. 8, 2000. Seilhamer, Jeffrey J.; Akerblom, Ingrid E.; Altus, Christina M.; Klingler, Tod M.; Russo, Frank; Au-Young, Janice; Hillman, Jennifer L.; Maslyn, Timothy J.: Database System Employing Protein Function Hierarchies For Viewing Biomolecular Sequence Data. van de Poll M L, Lafleur M V, van Gog F, Vrieling H, Meerman J H: N-acetylated and deacetylated 4'-fluoro- 4-aminobiphenyl and 4-aminobiphenyl adducts differ in their ability to inhibit DNA replication of single- stranded M13 in vitro and of single-stranded phi X174 in Escherichia coli. Carcinogenesis 13(5):751-8, (May) 1992. Vojtek A B, Hollenberg S M, Cooper J A: Mammalian Ras interacts directly with the serine/threonine kinase Raf. Cell 74(1):205-214, 1993. Wenzler H, Mignery G, Fisher L, Park W: Sucrose-regulated expression of a chimeric potato tuber gene in leaves of transgenic tobacco plants. Plant Mol Biol 13(4):347-54, 1989. White J S, White D C: Source Book of Enzymes. Boca Raton: CRC Press, 1997. Williams and Barclay, in Immunoglobulin Genes, The Immunoglobulin Gene Superfamily Winnacker E L. From Genes to Clones: Introduction to Gene Technology. VCH Publishers, New York, NY, .COPYRGT. 1987. Winter G and Milstein C: Man-made antibodies. Nature 349(6307):293-299, 1991. WO 00/04190; Filed Jul. 15, 1999, Published Jan. 27, 2000. Del Cardayre S, Tobin M, Stemmer W P, Ness J E, Minshull J, Patten P A, Subramanian V, Castle L A, Krebber C M, Bass S, Zhang Y, Cox T, Huisman G, Yuan L, Affholter J A: Evolution of whole cells and organisms by recursive sequence recombination. WO 00/09755; Filed Aug. 12, 1999, Published Feb. 24, 2000. Zarling D, Reddy G, Pati S: Domain specific gene evolution. WO 88/08453; Filed Apr. 14, 1988, Published Nov. 3, 1988. Alakhov J B, Baranov, V I, Ovodov S J, Ryabova L A, Spirin A S: Method of Obtaining Polypeptides in Cell-Free Translation System.

PGPUB-DOCUMENT-NUMBER: 20030143200

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030143200 A1

TITLE: Porcine adenovirus E1 region

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 963038

DATE FILED: September 24, 2001

US-CL-CURRENT: 424/93.2, 435/235.1 , 435/320.1 , 435/456 , 514/44

ABSTRACT:

The present invention relates to the characterization of the porcine adenovirus E1 region. The complete nucleotide sequence of the genome of porcine adenovirus type 3 (PAV-3), providing the characterization of the PAV3 E1 region, is described herein. Methods for construction of infectious PAV genomes by homologous recombination in procaryotic cells are provided. Recombinant PAV viruses are obtained by transfection of mammalian cells with recombinant PAV genomes. The PAV-3 genome can be used as a vector for the expression of heterologous nucleotide sequences, for example, for the preparation and administration of subunit vaccines to swine or other mammals.

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Pre-Grant Publication Document Identifier - DID (1):
US 20030143200 A1

Detail Description Paragraph - DETX (109):
[0138] Plasmid pPAV-200, which contains a full length PAV-3 genome, was generated by co-transformation of E. coli BJ 5183 recBC sbcBC (Hanahan, 1983, J. Mol. Biol. 166:557-580) with PstI-linearized pPAV-100 and the genomic DNA of PAV-3. Extensive restriction enzyme analysis of pPAV-200 indicated that it had the structure expected of a full-length PAV-3 insert, and that no unexpected rearrangements had occurred during recombination in E. coli.

Detail Description Paragraph - DETX (146):
[0159] In vitro Transcription and Translation

Detail Description Paragraph - DETX (165):
[0178] Characterization of PAV-3 .mu.l proteins In order to identify and characterize the proteins encoded by E1 region of PAV-3, anti-E1A, anti-E1B.sup.small and E1B.sup.large sera were produced by immunizing rabbits with 300 ug of gel purified GST-protein (glutathione S-transferase) fusions. Sera collected after the final boost was analysed by in vitro transcription and translation assays to determine specificity of the antibodies in the rabbit sera. The plasmids pSP64-PE1A, pSP64-PE1Bs and pSP64-PE1B1 were generated in

which coding sequence of E1A, E1B.sup.small and E1B.sup.large respectively, was placed downstream of the SP6 promoter (pSP64polyA vector containing SP6 promoter from Promega, Cat. No. P1241). In vitro translation of pSP64-PE1A RNA resulted in the synthesis of a polypeptide of 35 kDa (FIG. 10, lane 9), which was recognized by anti-E1A serum (FIG. 10, lane 7). In vitro translation of pSP64-PE1Bs RNA resulted in the synthesis of a polypeptide of 23 kDa (FIG. 10, lane 6) which was recognized by anti-E1B.sup.small serum (FIG. 10, lane 4). Similarly in vitro translation of pSP64-E1B1 RNA resulted in the synthesis of a polypeptide of 53 kDa (FIG. 10, lane 3), which was recognized by anti-E1B.sup.large serum (FIG. 10, lane 1). These proteins were not immunoprecipitated with anti-E1A serum (FIG. 10, lane 8), anti-E1B.sup.small serum (FIG. 10, lane 5) or anti-E1B.sup.large serum (FIG. 10, lane 2) from reactions in which pSP64polyA (negative control plasmid) was translated in vitro.

PGPUB-DOCUMENT-NUMBER: 20030099615

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030099615 A1

TITLE: Porcine adenovirus E1 and E4 regions

PUBLICATION-DATE: May 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 199550

DATE FILED: July 19, 2002

RELATED-US-APPL-DATA:

child 10199550 A1 20020719

parent continuation-in-part-of 09963038 20010924 US PENDING

US-CL-CURRENT: 424/93.2, 435/235.1 , 435/456 , 514/44

ABSTRACT:

The present invention relates to the characterization of the porcine adenovirus E1 and E4 regions. The complete nucleotide sequence of the genome of porcine adenovirus type 3 (PAV-3), providing the characterization of the PAV3 E1 region, is described herein. Methods for construction of infectious PAV genomes by homologous recombination in procaryotic cells are provided. Recombinant PAV viruses are obtained by transfection of mammalian cells with recombinant PAV genomes. The PAV-3 genome can be used as a vector for the expression of heterologous nucleotide sequences, for example, for the preparation and administration of subunit vaccines to swine or other mammals.

CROSS-REFERENCED TO RELATED APPLICATIONS

[0001] This is a continuation-in-part application of U.S. patent application Ser. No. 09/963,038, filed Sep. 24, 2001, which is incorporated by reference herein in its entirety.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030099615 A1

Detail Description Paragraph - DETX (109):
[0150] Plasmid pPAV-200, which contains a full length PAV-3 genome, was generated by co-transformation of E. coli BJ 5183 recBC sbcBC (Hanahan, 1983, J. Mol. Biol. 166:557-580) with PstI-linearized pPAV-100 and the genomic DNA of PAV-3. Extensive restriction enzyme analysis of pPAV-200 indicated that it had the structure expected of a full-length PAV-3 insert, and that no unexpected rearrangements had occurred during recombination in E. coli.

Detail Description Paragraph - DETX (150):
[0183] In Vitro Transcription and Translation

Detail Description Paragraph - DETX (174):
[0207] In order to identify and characterize the proteins encoded by E1 region of PAV-3, anti-E1A, anti-E1B.sup.small and E1B.sup.large sera were produced by immunizing rabbits with 300 ug of gel purified GST-protein (glutathione S-transferase) fusions. Sera collected after the final boost was analysed by in vitro transcription and translation assays to determine specificity of the antibodies in the rabbit sera. The plasmids pSP64-PE1A, pSP64-PE1Bs and pSP64-PE1B1 were generated in which coding sequence of E1A, E1B.sup.small and E1B.sup.large respectively, was placed downstream of the SP6 promoter (pSP64polyA vector containing SP6 promoter from Promega, Cat. No. P1241). In vitro translation of pSP64-PE1A RNA resulted in the synthesis of a polypeptide of 35 kDa (FIG. 10, lane 9), which was recognized by anti-E1A serum (FIG. 10, lane 7). In vitro translation of pSP64-PE1Bs RNA resulted in the synthesis of a polypeptide of 23 kDa (FIG. 10, lane 6) which was recognized by anti-E1B.sup.small serum (FIG. 10, lane 4). Similarly in vitro translation of pSP64-E1B1 RNA resulted in the synthesis of a polypeptide of 53 kDa (FIG. 10, lane 3), which was recognized by anti-E1B.sup.large serum (FIG. 10, lane 1). These proteins were not immunoprecipitated with anti-E1A serum (FIG. 10, lane 8), anti-E1B.sup.small serum (FIG. 10, lane 5) or anti-E1B.sup.large serum (FIG. 10, lane 2) from reactions in which pSP64polyA (negative control plasmid) was translated in vitro.

PGPUB-DOCUMENT-NUMBER: 20020168706

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020168706 A1

TITLE: Improved in vitro synthesis system

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

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APPL-NO: 10/ 091538

DATE FILED: March 7, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60273827 20010308 US

US-CL-CURRENT: 435/68.1, 435/91.2

ABSTRACT:

Compositions, systems, kits and methods relating to in vitro synthesis are provided. The system includes one or more extracts having reduced activity of an enzyme that catalyses hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds, an inhibitor that inhibits hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds, and/or at least two energy sources. The composition may include a nucleic acid template and one or more products of the nucleic acid template.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. provisional patent application Ser. No. 60/273,827, filed Mar. 8, 2001, the disclosure of which is specifically incorporated herein by reference.

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Pre-Grant Publication Document Identifier - DID
(1):
US 20020168706 A1

Summary of Invention Paragraph - BSTX (5):
[0005] In vitro protein synthesis has among its advantages specifically producing the desired protein without unnecessarily producing undesired proteins that are required for maintaining cells used for protein production in in vivo or cellular systems for protein synthesis. When a cell is used as a protein factory, in addition to producing the desired protein, the cell produces the other necessary molecules, including undesired proteins, that are required to maintain the cell. Cell-free systems are very popular because there are standard protocols available for their preparation and because they

are commercially available from a number of sources.

Summary of Invention Paragraph - BSTX (7):
[0007] Protein synthesis is guided by an RNA polynucleotide template that encodes the desired protein. Protein synthesis can be initially guided by a DNA template that is transcribed to produce the RNA polynucleotide that encodes the desired protein. The DNA template therefore minimally includes the DNA to be transcribed as well as a binding site for RNA polymerase that catalyzes transcription of the niRNA template that is translated to produce protein. When the transcription and translation are coupled in one system, the system is called an In Vitro Transcription Translation system (IVTT).

Summary of Invention Paragraph - BSTX (8):
[0008] IVTT or protein synthesis using cell-free extracts is becoming an important tool for analysis of proteins. The availability of complete genome sequences provides a wealth of information on the molecular structure and organization of a myriad of genes and open reading frames whose functions are not known or are only poorly understood. Thus, the utility of IVTT and more generally, protein synthesis in vitro, is expected to be even more important in the future for rapid and efficient protein synthesis and functional analysis.

Summary of Invention Paragraph - BSTX (22):
[0022] The problem of DNA template stability is especially evident when linear substrates, such as PCR derived products or restriction enzyme(s) digested fragments, are used in cell-free extracts for generating protein(s). The linear DNA fragments are susceptible to rapid degradation by intracellular exonucleases of E. coli, particularly RecBCD (Pratt et al, Nucleic Acids Res., 9: 4459-4474, (1981); Benzinger et al, J. Virol., 15: 861-871, (1975); Lorenz and Wackernagel, Microbiol Rev., 58, 563-602, (1994)) and possibly by other nucleases.

Summary of Invention Paragraph - BSTX (23):
[0023] In most cases, a supercoiled plasmid DNA containing the gene of interest is used in IVTT systems because plasmid DNAs are more stable (Kudlicki et al, Anal. Biochem., 206: 389-393, (1992)). Linear DNAs are more readably degraded by DNA nucleases, especially DNA exonucleases, such as RecBCD. Mutant RecBCD strains devoid of the exonuclease have been made. These mutant strains do not so rapidly degrade linear DNA; however, such mutant strains grow extremely poorly and therefore do not produce satisfactory results (Yu et al, PNAS, 97: 5978-5983, (2000)).

Summary of Invention Paragraph - BSTX (24):
[0024] E.coli extract for cell-free protein synthesis has been made using a RecD mutant of E.coli (Lesley et al, J. Biol. Chem., 266: 2632-2639, (1991)). However, cell-free extract made using RecD mutant E.coli contained high level of chromosomal DNA contamination because sheared chromosomal DNA is not degraded by the nuclease that has been mutated. To remedy this, micrococcal nuclease has been added to degrade the contaminating chromosomal DNA to minimize background. Similarly, entire RNase E deletion mutants have been made, but cell growth of these complete deletion mutants is also poor and unsuitable for providing a cell free extract.

Summary of Invention Paragraph - BSTX (28):
[0028] E.coli is known to contain a large number of RNases (Linn and Deutscher, In: Nucleases, Cold Spring Harbor Laboratory Press, 455-468, (1993)). RNase I, a periplasmic enzyme is one the major non-specific RNases in E.coli that acts on oligo RNA as a substrate (Meador et al, Eur. J. Biochem., 187: 549, (1990); and Meador and Kennel, Gene, 95: 1, (1990)). Several RNases participate in mRNA degradation in E.coli, including endonucleases (such as

RNase E, RNase K and RNase III) and 3'-exonucleases such as RNase II and polynucleotide phosphorylase (Gros et al, Nature, 190: 581-585, (1961); Emory et al, Genes Dev., 6: 135-148, (1992); Belasco, J. G., Control of mRNA stability, Academic Press, 3-12, (1993); Lopez et al, Mol. Microbiol., 33: 188-199, 1999; Mohanty and Kushner, PNAS, 97: 11966-11971, (2000)). The present invention is in part based on a premise that mutating or inhibiting these enzymes might therefore enhance protein synthesis in cell-free extracts.

Summary of Invention Paragraph - BSTX (34):

[0034] It has been shown in a wheat germ cell-free system that phosphatase-immunodepletion improved protein synthesis by reducing ATP and GTP hydrolysis (Kawarasaki et al, J. Biotech. 61: 199-208, (1998)). It has also been suggested that phosphoenolpyruvate (PEP), the substrate generally used for regeneration of ATP, is also degraded by phosphatase(s) limiting protein synthesis (Kim and Swartz, Biotech. and Bioeng., 66: 180-188, (1999); Swartz and Kim, U.S. Pat. No. 6,168,931 (2001); Kim and Choi, J. Biotech. 84: 27-32, (2000)).

Summary of Invention Paragraph - BSTX (39):

[0038] Compositions and methods are provided to enhance the synthesis with cell extracts. In one aspect, the cell providing the extract or components for synthesis is modified or mutated to inhibit or inactivate unwanted components/proteins/enzymes in the synthesis reaction. For example, mutations can be made in RNases, such as RNase E, or in other enzymes, such as alkaline phosphatase and endonuclease A in accordance with the present invention. In addition, inhibitors, such as inhibitors of nucleases that act on nucleic acid templates (e.g., Gam protein of phage lambda to inhibit RecBCD) or inhibitors of other unwanted or detrimental components/proteins/enzymes in the synthesis reaction can be used to enhance the production of desired products in vitro.

Summary of Invention Paragraph - BSTX (49):

[0048] Examples of nucleases that can be removed, inhibited, mutated, modified, or modulated according to the invention include: exonuclease I, exonuclease II, exonuclease III, DNA polymerase II, DNA polymerase III (subunit), exonucleases IVA and IVB, RecBCD (exonuclease V), exonuclease VII, exonuclease VIII, RecJ, dRpase, endonuclease I, endonuclease III, endonuclease IV, endonuclease V, endonuclease VII, endonuclease VIII, fpg, uvrABC, mutH, vsr endonuclease, ruvC, ecoK, ecoB, mcrBC, mcrA, mrr, and topoisomerases (such as topoisomerase I, topoisomerase II, topoisomerase III and topoisomerase IV). Such removal, inhibition, etc., allows preservation or protection of the nucleic acid template used in the synthesis reactions of the invention. For example, DNA nucleases of cells can be mutated, modified, inhibited, etc. to maintain or preserve the DNA templates. Such DNases from E.coli and other cells are known in the art.

Brief Description of Drawings Paragraph - DRTX (2):

[0056] FIG. 1 shows GamS-mediated inhibition of RecBCD activity.

Detail Description Paragraph - DETX (25):

[0085] RecBCD is an enzyme that has both single and double stranded exonuclease activity, single stranded endonuclease activity and helicase activity. The complex (and RecB in isolation) demonstrates ATPase activity. RecBCD is thus a nuclease and also a functional phosphatase.

Detail Description Paragraph - DETX (47):

[0106] In vitro cell-free protein synthesis using mutant E. coli

Detail Description Paragraph - DETX (48):

[0107] A mutant derivative of BL21 was used for cell-free protein synthesis in the IVTT system of the present invention. The starting BL21 strain was devoid of OmpT and Ion proteases (Studier, Methods in Enzymology, 185: 60-89, (1990)). The following genes were mutated: RNase I, the carboxy-terminus deletion of RNase E and endonuclease I by techniques known in the art. These mutations resulted in stabilizing or maintaining the DNA template and mRNA as well as stabilizing the proteins being synthesized. It has been reported that growth of E.coli cells in high phosphate containing media represses the synthesis of alkaline phosphatase (Malamy and Horecker, Biochemistry, 3: 1893-1897). Growing cells in high phosphate medium was therefore attempted to produce cells with low phosphatase activity. Additional mutations of other nucleases or other detrimental genes have can also be made to further enhancement (e.g., see below, Example 6)). The extracts made of from mutated E.coli grown in phosphate containing media enhance the production of proteins.

Detail Description Paragraph - DETX (51):
[0109] Instead of mutating the RecBCD, a novel method for inactivating the RecBCD nuclease has been found. The novel method involves incorporating lambda recombinase protein, Gam, in the cell-free extract.

Detail Description Paragraph - DETX (52):
[0110] Gam has been shown to inhibit E.coli RecBCD, in vivo, and has helped in homologous recombination using linear DNA (Yu et al, PNAS, 97: 5978-5983, (2000); Datsenko and Warner, PNAS, 97: 6640-6645, (2000)). In both cases, the authors demonstrated that, in the presence of lambda Gam, a linear DNA can be protected and the lambda Exo and Beta promote highly efficient homologous recombination. Gam was used here in the E. coli cell-free extract to attempt to protect linear DNA and thus potentially enhance protein and/or nucleic acid synthesis.

Detail Description Paragraph - DETX (55):
[0113] In addition, one report (Murphy, 1991) suggested that this short form of Gam, called GamS, had all of the RecBCD inhibitory activities of the in vivo gam gene product. For these reasons, we decided to clone the short form of Gam and try to express protein from it.

Detail Description Paragraph - DETX (60):
[0117] Activity Assay for Gam-mediated RecBCD inhibition

Detail Description Paragraph - DETX (61):
[0118] In order to assay Gam in vitro, we have developed a radioactive assay. Briefly, a double-stranded uniformly labeled linear DNA is used as the substrate for RecBCD activity utilizing purified RecBCD protein (Plasmid-Safe ExoV, Epicentre). Gam protein is preincubated with RecBCD and ATP, and then added to the DNA. After an incubation period, the mixture is bound to GFB filters, washed to remove small DNA fragments, and the counts remaining on the filter are determined. By comparing to the number of counts remaining using RecBCD without preincubation with Gam, the percent inhibition can be determined.

Detail Description Paragraph - DETX (62):
[0119] As shown in FIG. 1, 0, 1.5, or 30 units of GamS were incubated with 1 unit of purified RecBCD (Plasmid-Safe, Epicentre) in 100 .mu.l l.times.Plasmid-Safe buffer and 10 mM ATP for 10 minutes at 37.degree. C. 50 fmol of internally .sup.32P-labelled exonuclease substrate was added, and the reaction was continued at 37.degree. C. for 30, 60, 90, or 120 minutes. Reactions were stopped by the addition of 100 .mu.l filter binding buffer (6M guanidine, 100 mM MES) and kept on ice until all reactions were complete. Reaction mixtures (150 .mu.l) were spotted onto Millipore GF/B filters, washed

3.times.with 200 .mu.l 80% ethanol, dried, placed in 4 ml Scintisafe-F, and counted for 1 minute. GamS inhibits degradation of the substrate in a concentration dependent manner.

Detail Description Paragraph - DETX (71):
[0125] Stabilization of linear DNA against purified RecBCD and in E.coli cell-free extract in the presence of Gam

Detail Description Paragraph - DETX (72):
[0126] Analysis of E.coli cell-free extracts using the radioactive exonuclease assay showed that there are significant levels of RecBCD-like exonuclease activity in the extracts (approximately 0.4 units/.mu.l extract). Addition of purified GamS to the extract protected most of the substrate from degradation. However, 20% of the substrate was still degraded even at optimal levels of GamS. This suggests that the extract contains at least some other nucleases which are not inhibited by the presence of GamS. This result was further confirmed by experiments performed at longer incubation times. We have shown that linear DNA (PCR fragment) can be completely protected against purified RecBCD in the presence of GamS for incubation times up to 4 hours. (FIG. 1). However, in experiments involving GamS and crude extracts, lengthening the incubation time leads to the degradation of more substrate, independent of the levels of GamS added. After 30 minutes, 80% of the substrate remains; after 2 hours of incubation, only 30% of the substrate remains protected (FIG. 2). It is therefore likely that other E.coli nucleases, (such as ExoIII, ExoVIII, EndoIV and double stranded DNA specific nucleases) are also acting on the linear DNA with the result that full protection was not achieved. Therefore, mutations or inhibition of any or all of these genes are expected be useful to protect the template for longer periods of time.

Detail Description Paragraph - DETX (75):
[0128] Cell-free in vitro transcription-translation reactions (50 .mu.l) were prepared as described above using either a supercoiled plasmid DNA template or a PCR product template (50 ng). Each template encodes the CAT gene under the control of the T7 promoter. The PCR product was made directly from the supercoiled plasmid. GamS protein (200 ng) was added to E.coli extract and pre-incubated for 2 minutes at room temperature before addition to reactions. Reactions were carried out at 37.degree. C. for 2 hours. To quench reactions, RNase A (5ug) was added and reactions were incubated at 37.degree. C. for 15 minutes. Reactions were spotted (5 .mu.l) on GFC filters, washed 1.times.in 10% trichloroacetic acid, 2.times.in 5% trichloroacetic acid and 1.times.in methanol. Filters were dried, placed in 4ml Scintisafe-F, and counted for 1 minute. The amount (pmoles) of incorporated methionine for each sample is a measure of protein synthesis.

Detail Description Paragraph - DETX (76):
[0129] Cell-free protein synthesis was followed in the presence or absence of GamS protein. Supercoiled plasmid DNA or PCR products containing a CAT gene under the control of T7 promoter were used as substrates for protein synthesis. In the absence of Gam protein, yield from a PCR product DNA template is decreased (2-fold) as compared to the yield from a supercoiled DNA plasmid template. Addition of GamS protein to the reaction has no effect on synthesis from the supercoiled template. However, GamS restores protein production from the PCR product DNA template to a level comparable to that observed from the supercoiled DNA template (FIG. 3).

Detail Description Paragraph - DETX (81):
[0133] Recently, Lopez et al, (Mol. Microbiol. 33: 188-199, (1999)) reported that the protein synthesis per transcript in vivo could be increased

if an E.coli strain with a mutation in RNase E is used as a host for expression. In in vitro transcription-translation systems, the T7 promoter is often used in place of the native E. coli promoter and polymerase. Therefore, we tested if cell-free extract made from an RNase E mutant of E.coli might also enhance the synthesis of proteins using the different promoter system. Protein expression from four different plasmid templates, each containing the T7 promoter, was assayed using cell-free extract made from both an RNase E mutant strain and a wild type strain. Our results as described below suggest that cell-free extract of RNase E mutant E.coli enhanced protein synthesis up to 6 -fold compared to wild type E.coli (Table 1 and FIG. 4). It therefore appears that coordinating transcription, translation and enzymatic degradation can improve protein synthesis efficiency.

Detail Description Paragraph - DETX (82):

[0134] Cell-free in vitro transcription-translation reactions were carried out using three independent supercoiled plasmid DNA templates. These templates each encode a mammalian protein, Gus, v-Raf or tTak, under the control of the T7 promoter. Each template was tested for protein synthesis using extracts made from both the wild type (BL21-ERP) and the RNase E- (BL21-Star, Invitrogen Corp.) strains. Two amounts of each DNA template were assayed (500 ng and 100 ng). The amount (pmoles) of incorporated methionine is shown for each sample as a measure of protein synthesis. Fold increase in protein synthesis when using the RNase E-strain is shown to the right.

Detail Description Paragraph - DETX (83):

[0135] To test whether enhanced protein synthesis from the RNase E mutant strain is consistent over time, a time course experiment was carried out. Expression of two proteins, B-Gal and Gus, was assayed after 1, 2, 3 and 4 hours. Cell-free in vitro transcription-translation reactions (100 .mu.l) were prepared as described above using extract made from either the RNase E mutant strain BL21-Star (Invitrogen Corp, Carlsbad, Calif.) or a wild type strain (BL21-ERP). Reactions contain supercoiled plasmid DNA templates (1 .mu.g) encoding either the B-Gal or Gus gene under the control of the T7 promoter. Reactions were incubated at 37.degree. C. and aliquots (20 .mu.l) from each reaction were removed after 1, 2, 3, and 4 hours and quenched by treatment with RNase A. Aliquots were placed on ice until all reactions were complete. Samples were processed as described above in Example 7. The amount (pmoles) of incorporated methionine for each sample is a measure of protein synthesis.

Detail Description Paragraph - DETX (89):

[0140] Cell-free in vitro transcription-translation reactions were carried out using 750 ng of supercoiled plasmid DNA encoding the CAT gene driven by the T7 promoter. Acetyl phosphate was titrated into the reactions (0.1 mM to 60 mM) in the absence or presence of 30 mM PEP. Total reaction volume was 50 .mu.l. Reactions were incubated at 37.degree. C. for 2 hours. Reactions were quenched with RNase A and precipitated with trichloroacetic acid as described for FIG. 3. In this protocol, surprisingly, no inhibition of protein synthesis is observed at least up to 60 mM of acetyl phosphate (FIG. 5).

Claims Text - CLTX (28):

27. The in vitro synthesis system according to claim 1, wherein the at least one enzyme is selected from RecBCD and the at least one inhibitor is at least Gam.

Claims Text - CLTX (43):

42. The kit according to claim 41, comprising one or more of the components selected from the group consisting of: at least one inhibitor of RecBCD; at least one cell mutated at at least one gene selected from the group consisting of a nuclease, a polymerase and a phosphatase; at least one extract of a cell

mutated at at least one gene selected from the group consisting of a nuclease, a polymerase and a phosphatase; an inhibitor of at least one enzyme selected from the group consisting of a nuclease, a polymerase and a phosphatase; at least one energy source for synthesis; and a medium for growing said at least one cell.

PGPUB-DOCUMENT-NUMBER: 20020034559

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020034559 A1

TITLE: E. coli extract for protein synthesis

PUBLICATION-DATE: March 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ambuel, Yuping	Madison	WI	US	
Van Oosbree, Thomas R.	Madison	WI	US	
McCormick, Mark R.	Madison	WI	US	
Mierendorf, Robert C.	Madison	WI	US	

APPL-NO: 09/ 848449

DATE FILED: May 3, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60201450 20000503 US

US-CL-CURRENT: 424/780

ABSTRACT:

There is now a market for kits containing the necessary components for performing protein synthesis reactions using template DNA of the experimenter. One category of existing system for performing protein synthesis reactions is based on an S-30 extract from the bacteria E. coli. It is disclosed here that a simple fractionation process can dramatically improve the performance of an S-30 prokaryotic protein synthesis reaction mixture. In one embodiment, the fractionation is a simple freezing and thawing of an S-30 extract combined with a supplemental mix, followed by centrifugation. The resulting fractionated S-30 reaction mixture yields more full-length target protein and less non-full length or non-target protein than possible using prior art S-30 systems.

[0001] CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Application No. 60/201,450 filed May 3, 2000. This application is incorporated herein by reference in its entirety.

[0003] STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0004] Not applicable.

---------- KWIC --------

Pre-Grant Publication Document Identifier - DID
(1):
US 20020034559 A1

Summary of Invention Paragraph - BSTX (2):

[0005] The techniques of modern biotechnology have made possible the identification of the genetic elements, or genes, which control the characteristics of living organisms. The principle manner by which most genes cause effects in organisms is by encoding the construction of proteins. Thus in studying genes, it is often desired to produce a protein from the protein coding DNA from the gene, to study what the protein is, or what the protein does, or to perform some useful reaction with the protein. Sometimes a protein is expressed by inserting the entire gene, or an artificial construct carrying the protein coding sequence in an expression vector, into a suitable host cell so that the host cells can be grown to produce the protein. Another technique is to produce the protein in vitro directly from a gene or an artificial genetic construct in a cell free protein synthesis process. In vitro techniques for protein synthesis have the advantage that the protein can be produced directly from the encoding DNA without the necessity for intermediate culture and proliferation of transformed cells. In vitro protein synthesis provides the further advantage of allowing the production of proteins that are typically difficult or impossible to express in living cells, such as toxins or proteins containing amino acids that do not normally occur in living cells.

Summary of Invention Paragraph - BSTX (3):

[0006] Methods for in vitro transcription and translation of DNA to produce protein have been known for many years. The earliest documented descriptions of in vitro protein synthesis were developed in prokaryotic systems that utilized bacterial transcriptional and translational components to produce proteins in a coupled reaction. A common prokaryotic system, known as an E. coli S-30 cell free extract, was first described in a systematic way by Zubay, Annual Review of Genetics 7:267-287 (1973). Others have written articles and reviews on how to better make and use such S-30 extracts. In addition, kits for the in vitro production of proteins from DNA have been commercialized based on the use of S-30 extracts. Such kits are sold by several manufacturers. More recently, systems have been developed for transcription and translation using eukaryotic cell free extracts, particularly those based on the use of rabbit reticulocyte lysate or wheat germ extract. US Patent Nos. 5,324,637 and 5,895,753 describe systems for in vitro transcription and translation of protein.

Summary of Invention Paragraph - BSTX (4):

[0007] Both prokaryotic and eukaryotic cell free extracts for transcription and translation are sold today in commerce. In general, the researcher using an in vitro transcription and translation system wants the process to produce an optimum amount of the full length target protein and wants to minimize the amount of non-targeted protein and/or less than full length protein which is made. While prokaryotic systems are inherently simpler to use, the eukaryotic systems are thought to be superior for some applications. In particular, E. coli S-30 extracts are convenient to make and use but tend to produce a greater percentage of non-fill length protein than eukaryotic extracts. The production of unwanted protein or polypeptide products is generally observed by the presence of a variety of proteins different in size than the full-length target protein when the reaction products are visualized by gel electrophoresis. The non-full length proteins are thought to arise from several sources, which fall into two primary categories.

Summary of Invention Paragraph - BSTX (6):

[0009] The second major category of background in E. coli extracts results in the generation of smaller, truncated forms of the target protein. These forms arise from one or more causes, including: (1) initiation of protein synthesis at internal AUG start codons other than the authentic N-terminal AUG,

(2) synthesis of incomplete polypeptide chains due to premature termination of translation, (3) degradation of template DNA and/or RNA transcripts by nucleases present in the extract, and (4) degradation of the target protein by proteases present in the extract. The degradation of linear DNA templates has been approached by using extracts derived from strains deficient in one or more enzymes of the RecBCD complex (Yang et al., Proc. Natl. Acad. Sci. USA 77:7029-7033 (1980)). Strains deficient in ompT and lon proteases have also be used to minimize proteolytic degradation (Kohrer et al., Eur. J Biochem. 236:234-239 (1996)). While these examples appear to alleviate some degradation activity, there are many additional activities in cells that have not been possible to eliminate due to their being essential for cellular viability. In addition, there is not a method currently known in the art that generally addresses internal initiation or premature termination, which are believed to be significant causes of non-full length background. One may imagine that the use of stains deficient in one or more of the major ribonuclease activities present in E. coli may produce extracts exhibiting greater synthesis of full length proteins, but there have been no reports of success using this approach. The various RNA degradation pathways in E. coli and the interactions of enzymes and other proteins involved therein, both in vivo and in vitro, are still being elucidated.

Detail Description Paragraph - DETX (6):

[0030] Again, in accordance with the prior procedures, various manufacturers have made and marketed E. coli S-30 systems using the two separate constituents, S-30 extract and supplemental mix, which are combined only at the time of performing the protein synthesis reaction. Here it is contemplated that a combined fractionated S-30 reaction mixture will be sold as an item of commerce. The reaction mixture can be made by combining an S-30 E. coli extract and supplemental mix, freezing and thawing the combination, and centrifuging the result to remove particulate matter. The resulting fractionated reaction mixture is a clear, not cloudy, solution that can be pre-made, and stored until needed. This reaction mixture product can thus be distributed in containers ready for use in bulk or in aliquots for individual reactions or groups of reactions. This product can be stored and shipped frozen, or potentially dried or lyophilized and rehydrated prior to use. This product is a new item of commerce that would make performing protein synthesis reactions with prokaryotic cell-free extracts more efficient and convenient than with prior art systems. The fractionated S-30 reaction mix can be stored in suitable containers for frozen or dried storage and for shipment to users. The mix can be accompanied by instructions for use and with accompanying constituents, such as pretested water and a positive control DNA, to form kits, a common practice in the industry.

Claims Text - CLTX (24):

23. A method of making a reaction mixture for conducting a protein synthesis reaction in a prokaryotic cell free extract, the method comprising the steps of (a) making an E. coli S-30 extract by lysing E. coli cells and centrifuging the lysate; (b) separately, before or after step (a), making a supplemental mix including buffer salts, nucleotide triphosphates, an energy generating system, and precipitating agent that preferentially precipitates high molecular weight molecules; (c) combining the solutions of step (a) and (b); and (d) centrifuging the combined solutions and separating the supernatant to make the reaction mixture.

US-PAT-NO: 6355412

DOCUMENT-IDENTIFIER: US 6355412 B1

TITLE: Methods and compositions for directed cloning and subcloning using homologous recombination

DATE-ISSUED: March 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stewart; A. Francis	Leimen	N/A	N/A	DE
Zhang; Youming	Heidelberg	N/A	N/A	DE
Muyrers; Joep Pieter Paul	Meerssen	N/A	N/A	NL

APPL-NO: 09/ 350830

DATE FILED: July 9, 1999

US-CL-CURRENT: 435/4, 435/252.1 , 435/252.8 , 435/320.1 , 435/325 , 435/6 , 435/91.4 , 536/23.1

ABSTRACT:

The present invention is directed to methods and compositions for DNA subcloning using bacterial recombinase-mediated homologous recombination. The invention relates to methods for cloning, compositions comprising polynucleotides usefall as cloning vectors, cells comprising such polynucleotide compositions, and kits useful for cloning mediated by bacterial recombinases, such as RecE/T and Red.alpha./.beta..

54 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

US Patent No. - PN (1):
6355412

Detailed Description Text - DETX (86):
The host cell used for the cloning methods of the present invention and for propagation of the cloned DNA can be any cell which expresses the recE and recT and/or red.alpha. and red.beta. gene products, or any cell in which heterologous expression of these genes is possible. Examples of possible cell types that can be used include, but are not limited to, prokaryotic eukaryotic cells such as bacterial, yeast, plant, rodent, mice, human, insect, or mammalian cells. In a preferred embodiment, the host cell is a bacterial cell. In the most preferred embodiment, the host cell is an E. coli cell. Examples of specific E. coli strains that can be used are JC 8679 and JC 9604. The genotype of JC 8679 and JC 9604 is Sex (Hfr, F+, F-, or F'): F-.JC 8679 comprises the mutations: recBC 21, recC 22, sbcA 23, thr-1, ara-14, leu B 6, DE (gpt-proA) 62, lacY1, tsx-33, gluV44 (AS), galK2 (Oc), LAM-his-60, relA 1, rps L31 (strR), xyl A5, mtl-1, argE3 (Oc) and thi-1. JC 9604 comprises the same

mutations and further the mutation recA 56.

Other Reference Publication - OREF (15):
Jermutus L et al., "Recent advances in producing and selecting functional proteins by using cell-free translation", Curr Opin Biotechnol. Oct. 1998;9(5):534-48.

Other Reference Publication - OREF (26):
Murphy KC, "Lambda Gam protein inhibits the helicase and chi-stimulated recombination activities of Escherichia coli RecBCD enzyme", J Bacteriol. Sep. 1991;173(18):5808-21.

US-PAT-NO: 6255071

DOCUMENT-IDENTIFIER: US 6255071 B1
See image for Certificate of Correction

TITLE: Mammalian viral vectors and their uses

DATE-ISSUED: July 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Beach; David H.	Huntington Bay	NY	N/A	N/A
Hannon; Gregory J.	Huntington	NY	N/A	N/A
Conklin; Douglas	Huntington Bay	NY	N/A	N/A
Sun; Peiqing	Huntington	NY	N/A	N/A

APPL-NO: 08/ 820931

DATE FILED: March 19, 1997

PARENT-CASE:

This application is a Continuation-In-Part of application Ser. No. 08/716,926, filed Sept. 20, 1996 and now U.S. Pat. No. 6,025,192 which is incorporated herein by reference in its entirety.

US-CL-CURRENT: 435/69.1, 435/320.1 , 435/455 , 435/6 , 536/23.1 , 536/23.5 , 536/24.1

ABSTRACT:

The present invention relates to methods and compositions for the elucidation of mammalian gene function. Specifically, the present invention relates to methods and compositions for improved mammalian complementation screening, functional inactivation of specific essential or non-essential mammalian genes, and identification of mammalian genes which are modulated in response to specific stimuli.

In particular, the compositions of the present invention include, but are not limited to, replication-deficient retroviral vectors, libraries comprising such vectors, retroviral particles produced by such vectors in conjunction with retroviral packaging cell lines, integrated provirus sequences derived from the retroviral particles of the invention and circularized provirus sequences which have been excised from the integrated provirus sequences of the invention. The compositions of the present invention further include novel retroviral packaging cell lines.

58 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 23

---------- KWIC ---------

US Patent No. - PN (1):

6255071

Detailed Description Text - DETX (394):

The RK2 replication origin (oriV) requires a replication protein, trfA for function. Otherwise it is a silent DNA element thus allowing it to co-exist with a pUC replication origin on the same plasmid. The excised provirus depends on the RK2 origin for replication and thus for propagation of this plasmid, trfA must be provided in trans. Thus, a trfA-helper strain has been constructed using DH12S as a founder strain. Several characteristics of DH12S prompted its choice for construction of the helper strain. Firstly, it is defective in the restriction system that causes degradation of methylated DNA. Secondly, it is recA, recBC and will thus more stably maintain plasmids. Thirdly, it can be used for the production of single-stranded DNA. Finally, DH12S can give rise to high-efficiency electrocompetent cells.

Other Reference Publication - OREF (54):

Jang, S. et al., "A Segment of the 5' Nontranslated Region of Encephalomyocarditis Virus RNA Directs Internal Entry of Ribosomes During In Vitro Translation," J. Virol. (1988), vol. 62, No. 8, pp. 2636-2643.

US-PAT-NO: 6025192

DOCUMENT-IDENTIFIER: US 6025192 A

TITLE: Modified retroviral vectors

DATE-ISSUED: February 15, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Beach; David	Huntington Bay	NY	N/A	N/A
Hannon; Gregory J.	Huntington	NY	N/A	N/A

APPL-NO: 08/ 716926

DATE FILED: September 20, 1996

US-CL-CURRENT: 435/320.1, 435/6 , 435/DIG.24 , 536/23.1 , 536/23.5 , 536/24.1

ABSTRACT:

The present invention relates to methods and compositions for the elucidation of mammalian gene function. Specifically, the present invention relates to methods and compositions for improved mammalian complementation screening, functional inactivation of specific essential or non-essential mammalian genes, and identification of mammalian genes which are modulated in response to specific stimuli.

In particular, the compositions of the present invention include, but are not limited to, replication-deficient retroviral vectors, libraries comprising such vectors, retroviral particles produced by such vectors in conjunction with retroviral packaging cell lines, integrated provirus sequences derived from the retroviral particles of the invention and circularized provirus sequences which have been excised from the integrated provirus sequences of the invention. The compositions of the present invention further include novel retroviral packaging cell lines.

60 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

US Patent No. - PN (1):
6025192

Detailed Description Text - DETX (330):
The RK2 replication origin (oriV) requires a replication protein, trfA for function. Otherwise it is a silent DNA element thus allowing it to co-exist with a pUC replication origin on the same plasmid. The excised provirus depends on the RK2 origin for replication and thus for propagation of this plasmid, trfA must be provided in trans. Thus, a trfA-helper strain has been constructed using DH12S as a founder strain. Several characteristics of DH12S

prompted its choice for construction of the helper strain. Firstly, it is defective in the restriction system that causes degradation of methylated DNA. Secondly, it is recA, recBC and will thus more stably maintain plasmids. Thirdly, it can be used for the production of single-stranded DNA. Finally, DH12S can give rise to high-efficiency electrocompetent cells.

Other Reference Publication - OREF (45):

Jang, S. et al., 1988, "A Segment of the 5' Nontranslated Region of Encephalomyocarditis Virus RNA Directs Internal Entry of Ribosomes During In Vitro Translation", J. Virol. 62:2636-2643.

US-PAT-NO: 5385839

DOCUMENT-IDENTIFIER: US 5385839 A

TITLE: Transfer vectors and microorganisms containing human cytomegalovirus immediate-early promoter regulatory DNA sequence

DATE-ISSUED: January 31, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stinski; Mark F.	Iowa City	IA	N/A	N/A

DISCLAIMER DATE: 20091201

APPL-NO: 07/ 900056

DATE FILED: June 17, 1992

PARENT-CASE:

CROSS-REFERENCE TO PRIOR APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 07/582,130, filed Sep. 10, 1990, now U.S. Pat. No. 5,168,062; which was a continuation of U.S. patent application Ser. No. 07/256,134, filed Oct. 5, 1988, now abandoned; which was a continuation of U.S. patent application Ser. No. 07/058,662, filed May 22, 1987, now abandoned; which was a continuation of U.S. patent application Ser. No. 06/696,617, filed Jan. 30, 1985, now abandoned.

US-CL-CURRENT: 435/366, 435/252.3 , 435/252.33 , 435/320.1 , 435/69.1

ABSTRACT:

The cloning of a eucaryotic promoter-regulatory region that functions preferentially in human cells is disclosed. The invention is exemplified by the cloning of a section of the human cytomegalovirus genome comprising a DNA sequence with regulatory and promoter signals and an initiation site for RNA synthesis. The fragment, termed the human cytomegalovirus (HCMV) promoter-regulatory sequence, was obtained from purified HCMV DNA.

7 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

US Patent No. - PN (1):
5385839

Brief Summary Text - BSTX (26):
A novel plasmid designated pIEPR12 was produced as described above. A novel

microorganism, designated E. coli C600 RecBC.sup.- pIEPR12 was produced by transformation with pIEPR12. The novel microorganism and the novel plasmid were placed on deposit in the United States Department of Agriculture Northern Regional Research Center (NRRL) 1815 No. University Street, Peoria, Ill. 60604, on Jan. 30, 1985. The NRRL accession number for E. coli RecBC.sup.- pIEPR12 is B-15931. The accession number for plasmid pIEPR12 is B-15930.

Detailed Description Text - DETX (12):

The presence of the HCMV repeat sequences facilitates transcription both in vitro or in vivo. In vitro transcription was tested with a human cell lysate (HeLa cells) prepared by the method of Manley (Proc. Natl. Acad. Sci. U.S.A. 77:3855-3859, 1980). In vivo transcription was tested by inoculating human cells with calcium phosphate precipitates of the recombinant DNA, a method referred to as transfection, extraction of the cytoplasmic RNA, and measurement of the amount of specific RNA by a Berk and Sharp analysis (Cell 12:721-732, 1977) . Therefore, activation of a gene adjacent to the HCMV promoter-regulatory region is at the transcriptional level, i.e., the presence of these viral specific sequence increases the amount of transcription of the adjacent gene. There is ultimately a higher amount of mRNA available for translation in the cytoplasm. Therefore, it is proposed the HCMV regulatory sequences compete most effectively for cellular factors in the human cell that play a role in the transcription.

Detailed Description Text - DETX (23):

The plasmid designated pIEPR12 was used to transform bacteria E. coli C600 RecBC.sup.-. This bacterial host was selected because deletions would occur in E. coli HB101 RecA.sup.-. The transferred plasmid is replicated and propagated as the transformed microorganism replicates. As a result, large quantities of the HCMV promoter-regulatory region are duplicated. The microorganism passes on this capability to its progeny, so that in effect, the transfer has resulted in a new strain. The pIEPR12 recombinant DNA molecule consists of a transfer vector and the isolated HCMV viral DNA.

US-PAT-NO: 5168062

DOCUMENT-IDENTIFIER: US 5168062 A
See image for Certificate of Correction

TITLE: Transfer vectors and microorganisms containing human cytomegalovirus immediate-early promoter-regulatory DNA sequence

DATE-ISSUED: December 1, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stinski; Mark F.	Iowa City	IA	N/A	N/A

APPL-NO: 07/ 582130

DATE FILED: September 10, 1990

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. application Ser. No. 07/256,134 filed Oct. 5, 1988, now abandoned, which was a continuation of prior application Ser. No. 07/058,662, filed May 22, 1987 (abandoned), which was a continuation of prior application Ser. No. 06/696,617, filed Jan. 30, 1985 (abandoned).

US-CL-CURRENT: 435/366, 435/252.3 , 435/252.33 , 435/320.1

ABSTRACT:

The cloning of a eucaryotic promoter-regulatory region that functions preferentially in human cells is disclosed. The invention is exemplified by the cloning of a section of the human cytomegalovirus genome comprising a DNA sequence with regulatory and promoter signals and an initiation site for RNA synthesis. The fragment, termed the human cytomegalovirus (HCMV) promoter-regulatory sequence, was obtained from purified HCMV DNA.

8 Claims, 3 Drawing figures

Exemplary Claim Number: 1,4

Number of Drawing Sheets: 3

---------- KWIC ---------

US Patent No. - PN (1):
5168062

Brief Summary Text - BSTX (25):
A novel plasmid designated pIEPR12 was produced as described above. A novel microorganism, designated E. coli C600 RecBC.sup.- pIEPR12 was produced by transformation with pIEPR12. The novel microorganism and the novel plasmid were placed on deposit in the United States Department of Agriculture Northern

Regional Research Center (NRRL) on Jan. 30, 1985. The NRRL accession number for E. coli RecBC.sup.- plEPR12 is B-15931 The accession number for plasmid plEPR12 is B-15930.

Detailed Description Text - DETX (12):

The presence of the HCMV repeat sequences facilitates transcription both in vitro or in vivo. In vitro transcription was tested with a human cell lysate (HeLa cells) prepared by the method of Manley (Proc. Natl. Acad. Sci U.S.A. 77:3855-3859, 1980). In vivo transcription was tested by inoculating human cells with calcium phosphate precipitates of the recombinant DNA, a method referred to as transfection, extraction of the cytoplasmic RNA, and measurement of the amount of specific RNA by a Berk and Sharp analysis (Cell 12: 721-732, 1977) Therefore, activation of a gene adjacent to the HCMV promoter-regulatory region is at the transcriptional level, i.e., the presence of these viral specific sequence increases the amount of transcription of the adjacent gene. There is ultimately a higher amount of mRNA available for translation in the cytoplasm. Therefore, it is proposed the HCMV regulatory sequences compete most effectively for cellular factors in the human cell that play a role in the transcription.

Detailed Description Text - DETX (23):

The plasmid designated plEPR12 was used to transform bacteria E. coli C600 RecBC.sup.-. This bacterial host was selected because deletions would occur in E. coli HB101 RecA.sup.-. The transferred plasmid is replicated and propagated as the transformed microorganism replicates. As a result, large quantities of the HCMV promoter-regulatory region are duplicated. The microorganism passes on this capability to its progeny, so that in effect, the transfer has resulted in a new strain. The plEPR12 recombinant DNA molecule consists of a transfer vector and the isolated HCMV viral DNA.

Claims Text - CLTX (8):

8. E. coli RecBC.sup.- plEPR12.